REMARKS

The applicant has amended the claims as set out above and provides the remarks as set out below to address each concern raised by the office in the official communication mailed December 18, 2002.

Cancellation of Claims (Item 1). Claims 1-14 and 70-135 as amended under Article 34, PCT have been canceled without prejudice by the preliminary amendment filed January 29, 2001. The applicant without prejudice formally cancels claims 15-44, 52-59, and 61-69 to non-elected Groups I, II, III, V, VI set out in the restriction mailed April 24, 2002. The applicant also cancels claim 60. The applicant does not waive any right to have these claims examined without any reduction in claim breadth in a subsequent continuing application, if desired. The applicant respectfully requests entry and examination of remaining claims 45-51 and newly added claims 136 and 137.

Supplemental Information Disclosure (Item 2). The applicant resubmits the non-English reference of Linge et al including a concise statement of relevance as required under 37 C.F.R. §1.98(a)(3).

Section 112 Concerns (Item 3). The office raised concerns under §112, second paragraph with respect to claims 45-51 and 60.

The applicant has without prejudice canceled claim 60 and the §112 concerns with respect to this claim are moot.

Claim 45. The applicant has amended 45 to make clear how collected equine sperm cells and staining of equine sperm cells are related to the step of establishing a cell source. The applicant has set out the definition of "cell source" in the disclosure starting on page 13, l. 25 as follows:

"The flow cytometer instrument includes a sample input, here an equine sperm cell source (1) which acts to establish or supply equine sperm cells or some other type of item to be analyzed by the flow cytometer."

Since the applicant has provided an explicit definition of "cell source" in the disclosure it is proper for the applicant to use the term in the claims without amendment. MPEP §2106 (C); Markman v. West view Instruments, 52 F.3d 967, 980, 34 USPQ2d 1321, 1330 (Fed. Cir.)(en banc), aff'd 116 S.Ct. 1384 (1996). The applicant's definition should control interpretation as it is used in the claim." MPEP, §2106(C); Toro Co. v. White Consolidated, 199 F.3d 1295, 1301, 53 USPQ 1065, 1069 (Fed. Cir. 1999).

As such, the applicant respectfully requests that term "cell source" as recited remain in claim 45 as amended.

The applicant has also amended claim 45 to make clear the relationship between "staining" and the step of discriminating between equine cells based on their "sex characteristic".

The applicant has also amended the recitation of "having the desired sex characteristics" in claim 45 to recite "collecting said equine sperm cells separated based upon said sex characteristic in said skim milk solution" to address the antecedent basis concern.

Claim 49-51. The applicant has deleted the recitation of "high speed flow cytometry" and "high speed cell sorter" to address the office's concern with clarity.

Section 103 Concerns. With respect to claims 45-47, 49-51 and 60, the office raised §103 concerns as being unpatentable over United States Patent No. 5,985,216 to Rens ("Rens") in view of Cryobiology 33;320-329 (1996) authored by Wilhelm ("Wilhelm").

The applicant has amended claim 45 as set out in the clean version of the claims to further include element i. which recites the step of "establishing a equine artificial insemination sample containing at least some of said equine sperm cells separated based upon said sex characteristic which are capable of fertilizing at least one egg within a female of said species of equine mammal." which further clarifies the differences between the claimed invention and the combination of references.

First, the applicant specifically traverses the office's indication on page 5 of the official communication that Rens teaches that "the sperm is stained with Hoeschst 33342 dye in order to distinguish between viable and nonviable sperm." The applicant has reviewed the Rens reference at the citation provided by the office (Col. 5, lines 4-10) and does not see any teaching that Hoeschst 33342 dye is used to distinguish between viable and nonviable sperm. The Rens reference states "Sperm were subsequently stained with 7.1 uM Hoechst 33342 per 15 x 10^6 sperm (Calbiochem-Behring Corp., La Jolla, Calif.) and incubated over a 40-min period at 32°C." This statement does not any manner teach that sperm cells can be stained to differentiate viable from non-viable sperm cells. As can be understood by the Rens reference, the fluorochrome [Hoeschst 33342] of the stained sperm is used so that it can be excited by ultraviolet light to differentiate between X and Y sperm. Rens, Col. 5, lines 38-39.

Similarly, the applicant specifically traverses any indication by the office that the statement by Rens at Col. 5, lines 8-10 that "For bull sperm studies, just prior to analysis propidium iodide (PI) was added to the Hoechst 33342 stained sperm. This allowed dead sperm to be distinguished from living sperm as described by Johnson et al. (1994, supra)" teaches staining to "distinguish between viable and nonviable sperm".

It is well known that a "living sperm cell" is not necessarily a "viable sperm cell". For example, as pointed out by the Rens reference, the "beveled needle helps to orient sperm especially sperm heads, e.g., sperm without their tail." Rens, Col. 1, lines 62-63. Sperm heads can be "living sperm cells" but they are cannot be viable sperm cells. Rens, Col. 6, lines 26-29 (comparing motile sperm cells, sperm heads, and dead sperm cells).

The applicant also specifically traverses the indication by the office that "approximately 50% of the sperm were viable". Official Communication at page 5. Rens only compares motile sperm cells, non-motile sperm heads, and dead sperm cells for the practical purpose of showing that the nozzle is capable of orienting sperm cells which move to approximately the same extent that the nozzle orients non-moving sperm cells (tailess or dead). Rens, Col. 6, lines 26-29. This provides a "showing that motility has a negligible influence on orientation of bull sperm when the novel elliptical nozzle is used." Rens, Col. 6, lines 38-40. Rens does not teach that the sorted subpopulations of X and Y sperm cells or any proportion of the sorted subpopulations are dead sperm cells, motile sperm cells, viable sperm cells, or fertile sperm cells which is the typical definition of "viable sperm cells".

The applicant also specifically traverses any indication by the office that the limitation of "sort rates" as claimed by the applicant are the same as the indication by the office that "sorting was performed at sampling rates of 500 sperm /sec and 2000 sperm/sec". Rens does not teach that sperm cells are sorted into X and Y subpopulations at 500 sperm/sec or are sorted at 2000 sperm/sec. Rens teaches "sample rates of 500 sperm/sec. and 2000/sec." Rens, Col. 6, line 48. The "sample rates" taught by Rens reflect the number fluorescent events analyzed each second. The number of fluorescent events generated each second is adjusted by increasing the flow rate of the sheath fluid for a given number of sperm cells introduced or increasing the numbers of sperm cells introduced into the sheath fluid. In either case, Rens samples all or a portion of these fluorescent events to compare orientation of sperm cells at varying numbers of fluorescent events. As indicated by the office, the sample rate as defined Rens can be up to 15,000/sec. Rens, Col. 4, lines 29-31. The "sample rate" however as taught by Rens is entirely different than actually "obtain[ing] approximately 1000 live sperm cells . . .of each sex chromosomal composition" as described and claimed by the applicant. Specification, page 24, lines 3-5, claim 51.

The applicant also specifically traverses any indication by the office that the "equine sperm cells separated based upon said sex characteristic which are capable of fertilizing at least

one egg within a female mammal" as claimed are the same as the "spermatozoa" taught by the Willhelm.

While it is easily overlooked, separated sexed spermatozoa are not naturally occurring sperm cells. First, regardless of the method of sexing, sexed sperm cells are removed from the natural chemical environment of the seminal fluids which support motility, viability, and fertility, either by dilution or by introduction into sheath fluid. Second, sexed sperm cells are modified in some fashion, such as by staining which binds to the nuclear DNA. Third, sexed sperm cells are diluted in a manner that reduces sperm cell functionality. Fourth, sexed sperm cells have been processed in a manner that subjects the sperm cells to unnatural stresses (pressure, dilution, staining, acceleration, deceleration, salt concentrations, impact, mechanical transfer, exposure to laser light, etc.). Specification starting at page 16, line 13. It is well known that these non-naturally occurring sperm cells have very low vigor when conventionally sorted. By comparison, the "spermatozoa" taught by Wilhelm are naturally occurring spermatozoa which are treated in an entirely different manner than the method claimed. Wilhelm does not teach staining of the spermatozoa to allow differentiation based upon sex but only stains to determine permeabilization of the plasma membrane. Nor does Wilhelm teach separation of equine sperm cells into subpopulations.

As indicated by the office the Catt reference does not teach the use of HEPES buffered medium as a sheath fluid.

As such, the combination of the references do not disclose all the steps of the claimed invention as required by §2143, MPEP; §2143.03, MPEP; In re Royka, 490 F.2d 981 (CCPA 1974). The applicant believes that the following steps are not taught:

- "staining said equine sperm to allow differentiation based upon a sex characteristic" or "differentiating between said equine sperm cells entrained in said droplets based upon said sex characteristic" or "separating said droplets based upon said sex characteristic of said equine sperm cells entrained". Claim 45, elements b, f, and g. The combination

of references does not teach any aspect of staining, analysis, or sorting of equine sperm cells as the office indicates.

- establishing a skim milk solution into which said droplets are separated based upon said sex characteristic. Claim 45, element h. The combination of references does not teach the use of skim milk solution in which stained and sorted equine sperm cells are collected as the office indicates.

- establishing an equine artificial insemination sample containing at least some of said equine sperm cells separated based upon said sex characteristic which are capable of fertilizing at least one egg within a female of said species of mammal. Claim 45, element i.

The combination of references do not teach any sorted sperm cells are fertile sperm cells nor do the references teach even a single pregnancy of any mammal using sorted sperm cells. By comparison the disclosure states "In summary, this invention has demonstrated for the first time, that pregnancy in the mare can be achieved, and foals of predetermined sex can be obtained. . ." Specification, page 40, lines 16-17.

- wherein said sheath fluid contains HEPES buffered medium. Claim 48.

- sorting said droplets having said equine sperm cells entrained at a rate of at least nine hundred per second. Claim 50.

- operating said flow cytometer at a pressure of at least fifty pounds per square inch.

- an equine artificial insemination sample of no more than five million sperm cells, an equine insemination artificial insemination sample of no more than twenty five million sperm cells. Claim 136.

- establishing said artificial insemination sample in a volume selected from the group of 0.2 mL and 1mL.

Because none of the combinations of references cited by the office disclose, teach or suggest the various limitations of the invention claimed by the applicant as set out above, a prima facie case of obviousness cannot be established with regard to these claim limitations, nor any claim depending there from. §21243.03, MPEP and In Re Fine, 837 F.2d 1071 (Fed. Cir. 1988). As such, the applicant believes that claims 45-51 and claims 136 and 137 as amended are now in condition for allowance, and the applicant respectfully requests an allowance of same.

Also the combination of references cited by the office do not provide the requisite suggestion or motivation to combine the references as required by §2143.01, MPEP. The mere fact that references can be combined does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination. §2143.01, MPEP; In Re Mills, 916 F.2d 680 (Fed. Cir. 1990). Simply because combining the elements is well within the ordinary skill of the art at the time of the claimed invention is not sufficient to establish a prima facie case of obviousness. §2143.01, MPEP; Ex Parte Levengood, 28 USPQ2d 1300 (Bd. Pat. App & Inter. 1993). Also, if the proposed combination of the prior art would change the principle of operation of the prior art then the teachings of the references are not sufficient to render the claims obvious. §2143.01, MPEP and In Re Ratti, 270 F.2d 810 (CCPA 1959).

As discussed above, the use of "a milk solution" in which sorted sperm cells are collected is not disclosed by the combination of references. Moreover, the compositions taught are by Wilhelm are not the same as claimed by the applicant. The Wilhelm compositions contain glycerol for the purpose of freezing spermatozoa while the "skim milk solution" claimed by applicant does not contain glycerol. There is no suggestion in the combination of references that a solution used for cryopreservation purposes such as the "tyrodes-based skim milk-egg yolk extender" could or should be used as a solution in which to collect sorted spermatozoa for artificial insemination.

Similarly, no combination of the references suggests that HEPES-buffered synthetic oviduct fluid taught by Catts should be used for the entirely different purpose as a flow cytometer sheath fluid. The viability of ram spermatozoa in HEPES as shown by the Catt reference is actually lower than in the PBS which is typically used for sheath fluid. Catt reference, Figure 1 (d).

Also, no combination of references suggest that a flow cytometer should be operated at 50 psi as claimed. Claim 51. Because the applicant discloses the first equid resulting from artificial insemination with sorted equine sperm cells this result is unexpected. The higher operating pressure allows sort rates of 900 sperm cells per second to be achieved which allows an artificial equine insemination sample to be established before all the sperm cells are dead or lack viability necessary to fertilize eggs in vivo. Based on the sort rates disclosed by the combination of references even a low dose equine insemination sample of 25,000,000 equine sperm cell insemination sample would take about 34 hours to sort. Understandably at 34 hours the majority of equine sperm cells in the collected sample would be dead.

Because the Rens, Wilhelm, and Catt references do not provide the requisite suggestion or motivation to be combined as required, the applicant respectfully requests an allowance of claims 45-51 and claims 136 and 137.

Also, the combination of references do not provide one of ordinary skill in the art a reasonable expectation of successfully making the invention at the time the invention was made. §2143.02, MPEP. Where the prior art provides only a general approach as to the particular form of the claimed invention or how to achieve it, the invention is not obvious, but only obvious-to-try. In Re O'Farrell, 853 F.2d 894, 903 (Fed. Cir. 1988).

The combination of references does not provide any specific approach to establishing an "equine artificial insemination sample. . .capable of fertilizing at least one egg in vivo." As discussed above, various limitations claimed by the applicant are entirely lacking in the combination of references. The office attempts to modify the Rens teaching with additional

teachings from Wilhelm and Catt which as discussed above are not suggested by the references themselves because the compositions disclosed are different then those claimed or used for entirely different purposes. Importantly, the combination of references does not disclose even a single pregnancy of any mammal from insemination with sorted spermatozoa. This true even for cows for which at least 10 fold fewer fertile sperm are typically used for artificial insemination than for equids.

Because the combination of the Rens, Wilhelm, and Catt references do not provide a reasonable expectation of successfully making the invention as required, the applicant respectfully requests an allowance of claims 45-51 and claims 136 and 137.

Finally, the Applicant notes that the Catt reference is within one year of the priority date and may be withdrawn from examination through the use of a 1.131 affidavit.

VERSION WITH MARKINGS TO SHOW CHANGES MADE

Claims 1-14 and 70-135 as amended under Article 34, PCT have been canceled without prejudice by the preliminary amendment filed January 29, 2001.

Claims 15-44, have been formally canceled by the applicant without prejudice.

45 A method of sorting equine sperm cells according to a determination of their sex characteristic comprising the steps of:

a. [collecting] obtaining equine sperm cells from a male of a species of [an] equine mammal;

b. staining said equine sperm cells to allow differentiation based upon a sex characteristic;

c. establishing a cell source which [supplies] introduces said equine sperm cells which have been stained [to be sorted] into a sheath fluid;

d. [establishing] forming droplets in said [a] sheath fluid [which is adapted to form droplets and which is compatible with said equine sperm cells];

e. entraining said equine sperm cells which have been stained in said droplets; [establishing a skim milk solution into which said equine sperm cells are collected;]

f. [discriminating] differentiating between said equine sperm cells entrained in said droplets [according to a determination of their] based upon said sex characteristic;

[g. entraining individual equine sperm cells in a droplet;]

[h] g. [sorting] separating said droplets [according to] based upon said sex characteristic of [the] said [individual] equine sperm cells entrained [they contain]; [and]

[i] h. establishing a skim milk solution into which said droplets separated based upon said sex characteristic of said equine sperm cells entrained are collected

i. collecting said equine sperm cells [having the desired] separated based upon said sex characteristic in said skim milk solution;

j. establishing a equine artificial insemination sample containing at least some of said equine sperm cells separated based upon said sex characteristic which are capable of fertilizing at least one egg within a female of said species of equine mammal.

46 A method of sorting equine sperm cells according to a determination of their sex characteristic as described in claim 45 wherein said step of establishing a skim milk solution into which said equine sperm cells are collected comprises the step of establishing a solution containing a skim milk extender as a collection fluid.

47 A method of sorting equine sperm cells according to a determination of their sex characteristic as described in claim 46 wherein said step of establishing a skim milk solution into which said equine sperm cells are collected further comprises the step of establishing a solution containing about four percent egg yolk as a collection fluid.

48 A method of sorting equine sperm cells according to a determination of their sex characteristic as described in claim 45 wherein said [step of establishing a sheath fluid which is adapted to form droplets and which is compatible with said equine sperm cells comprises the step of establishing a] sheath fluid contain[ing]s a [hepes] HEPES buffered medium.

49 A method of sorting equine sperm cells according to a determination of their sex characteristic as described in claim 45 wherein said step of [sorting said droplets according to said sex of the individual equine sperm cells they contain] separating said droplets based upon said sex characteristic of said individual equine sperm cells comprises the step of sorting [according to said sex of] said droplets having said equine sperm cells entrained [through the use of high speed] using a flow cytomet[ry] er.

50 A method of sorting equine sperm cells according to a determination of their sex characteristic as described in claim 49 wherein said step of sorting [according to said sex

of said equine sperm cells through the use of high speed] said droplets having said equine sperm cells entrained using a flow cytomet[ry]er comprises the step of [collecting live] sorting said droplets having said equine sperm cells entrained [sperm of the desired sex] at [the] a rate of at least nine hundred [live sperm] per second.

51 A method of sorting equine sperm cells according to a determination of their sex characteristic as described in claim 49 wherein said step of sorting [according to said sex of said equine sperm cells through the use of high speed flow cytometry] said droplets having said equine sperm cells entrained comprises the step of operating [a high speed] said flow cytometer [cell sorter] at a pressure of at least about fifty pounds per square inch.

Claims 52-59 have been formally canceled by the applicant without prejudice.

[60 A method of flow cytometry accomplished through use of a method as described in any of claims 45, 48, or 50.]

Claims 61-69, have been formally canceled by the applicant without prejudice.

--136. A method of sorting equine sperm cells according to a determination of their sex characteristic as described in claim 45, wherein said equine artificial insemination sample is selected from the group consisting of: an equine artificial insemination sample of no more than about five million equine sperm cells, and an equine artificial insemination sample of no more than about twenty-five million equine sperm cells.

137. A method of sorting equine sperm cells according to a determination of their sex characteristic as described in claim 45, wherein said equine artificial insemination sample has a volume selected from the group consisting of : 0.2 ml, and1ml.--

CONCLUSION

The applicant has formally canceled claims 15-44, 52-59, and 61-69. The applicant has addressed the Section 103 concerns and Section 112 concerns raised by the office. The applicant believes that claims 45-51 and newly added claims 136 and 137 are now in condition for allowance and the applicant respectfully requests an allowance at the examiner's earliest convenience.

Dated this 18th day of June, 2003.

Respectfully submitted,

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